Coding potential of transfected human placental lactogen genes

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Received June 28, 1990; Revised and Accepted July 16, 1990

ABSTRACT

We have joined the promoter-less sequences of the three hPL genes (hPL-1, hPL-3 and hPL-4) to strong transcriptional control elements. *in vivo* ^{35}S-labeled proteins from the culture medium of cells transfected with the genes were resolved on SDS-polyacrylamide gels. The presence of characteristic labeled bands, visualized by autoradiography, determined that hPL-4 and hPL-3, but not hPL-1, contribute to the production of mature hPL. In these experiments hPL-3 expressed more RNA and protein than hPL-4. By exchanging the first two exons among hPL and hGH genes, we determined that the abundance of chimeric proteins depended on the genetic origin of the first two exons. Finally, we found evidence indicating that the splice mutation (G→A) at the beginning of the second intron of hPL-1, is not the only cause of the apparent lack of inactivity of this gene, since its reversion does not restore expression.

INTRODUCTION

The human growth hormone-placental lactogen gene family is a multigene complex containing two human growth hormone (hGH) and three human placental lactogen (hPL, also known as chorionic somatommamotropin; hCS) genes (1,2). The entire gene cluster is located on the long arm of human chromosome 17 at bands q22−24 (3). The genes display the following 5′ to 3′ arrangement: hGH-N, hPL-1 (or hPL-L, L for like), hPL-4 (also known as hCS-A or hCS-1), hGH-V and hPL-3 (also named hCS-B or hCS-2). The best characterized protein products of this family are the secreted hGH and hPL polypeptides containing 191 amino acids. They are produced in the pituitary gland and placenta, respectively.

Recombinant DNA analysis has revealed a paradox in the coding potential of the hGH and hPL genes. The hGH-N gene, both *in vivo* and *in vitro*, generates through differential splicing of its primary transcription product, 22 kDa (90%) and 20 kDa (10%) forms of hGH (4,5). The hGH-V gene, whose expression has been demonstrated only in the placenta and in a single human pituitary tumor (6), has recently been confirmed to also generate *in vitro* a 22 kDa form. However, no 20 kDa protein derived from this gene has been detected (7). cDNA cloning and DNA sequencing also have revealed the existence in placenta of a second type of hGH-V mRNA. By retaining an in-frame fourth intron, this new mRNA is predicted to encode a mature protein of 26 kDa (8).

A completely different situation is observed with the hPL genes. The hPL-4 and hPL-3 genes have been found to be active in term placenta. In addition, their cDNAs have been cloned and sequenced (9). Their mRNAs are slightly divergent in nucleotide sequence. The encoded pre-hormones of these two mRNAs differ in a single amino acid position within the signal peptide (at position −24, hPL-3 codes for alanine while hPL-4 codes for proline). Yet, the mature hormones are identical. The third gene (hPL-1), is presumably nonfunctional, since it contains a mutation (G→A) at the 5′ or donor splice site of the second intron. Transcripts derived from it have not been detected (9, 10). Therefore, while two hGH genes generates at least four different hormones, the sequences of the three hPL genes predict the synthesis of a single form of mature hPL hormone.

The sequence of both hPL-3 and hPL-4 genes and their cDNAs, predict that they might contribute to the placental production of hPL. However, since the mature proteins expected to be derived from them are identical, it is impossible to distinguish their gene(s) of origin. No evidence has been obtained demonstrating that the expression of either gene actually specifies the mature hPL protein.

In this study we performed an analysis of the *in vitro* expression products of all hPL genes. We specifically addressed the question of whether or not the hPL-3 and hPL-4 genes, known to be transcriptionally active in term placenta, produce mature hormones. These two genes, at different expression levels, were found capable of producing an intrinsic hPL protein. Experiments were also designed to determine if the splice point mutation at the beginning of the first intron of the hPL-1 gene, is the only cause of its apparent lack of expression.

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MATERIALS AND METHODS

Recombinant DNA constructions and preparation of plasmid DNA

Restriction and other enzymes were obtained from commercial suppliers and used according to their manufacturers instructions. The isolation of hGH and hPL genes have been previously reported (11). pNUT, constructed by R. Palmiter *et al.* (12) which already carries the hGH-N structural gene in front of the metallothionein promoter, was a generous gift. The hPL genes cloned in pSV2gpt (13) were kindly provided by G. Saunders. DNA restriction fragments were purified from preparative agarose or polyacrylamide gels. This was performed by electroelution, or by glass bead extraction (14) from the agarose gel slices. hPL and hGH promoter-less genes were subcloned into pNUT. The large *Bam*HI to *Eco*RI fragment of pNUT, was ligated to DNA fragments carrying the genes of interest. The genes consisted of sequences from their naturally occurring *Bam*HI site (except hPL-1; see Results) at nucleotide +2, to a natural or artificial (linkers) *Eco*RI site located several hundred nucleotides downstream of the polyadenylation signal. To construct our negative control, pNUT(−), we took advantage of the presence, in pNUT, of two *Xma*I sites. They flank hGH coding sequences: one artificial site is present at position −4, while the other is a natural site located four nucleotides downstream of the termination codon. By cutting with *Xma* I, diluting and ligating back, we obtained the derivative of pNUT lacking the hGH structural gene: pNUT(−).

Ligations, bacterial transformations and plasmid DNA isolation and characterization were carried out using standard protocols (15). Recombinant plasmids carrying all hGH, hPL or hybrid genes were characterized by digesting their DNAs with several diagnostic enzymes, by Southern blotting (16) or nucleotide sequencing (17).

Cell culture, DNA transfection, isolation of RNA and labeling of secreted proteins

COS-7 cells (a gift from T. Kuo) were adapted to grow in Dulbecco's modified Eagle's medium (Sigma chemical Co, St Louis MO.) containing 1% fetal calf serum (FCS), (Hyclone Laboratories, Inc. Logan, Utah). They were maintained at 37°C with 5% CO_2. By lowering the FCS concentration we could precipitate and analyze larger volumes of media. Plasmid DNA (7.5 μg/ 25 cm^2 culture flask) was transfected by the calcium phosphate method (18). We evaluated transfection efficiency performing CAT assays or through radioactivity counting of RNA hybridizated with the DHFR probe in slot blots. The CAT assays were carried out on a fraction of cultured cells or the entire culture co-transfected with both the test plasmid and pCMVCat (19).

Total RNA was recovered by the guanidinium thiocyanate-phenol-chloroform technique (20). Quantity and quality of RNA preparations were determined spectrophotometrically and corroborated by agarose gel electrophoresis (15).

To label newly synthesized and secreted proteins, 48 h after cells transfection the previously mentioned medium was replaced for a methionine-free medium containing 1% dialyzed FCS and ^{35}S-methionine (Amersham Intl, Buckinghamshire, England). *In vivo* labeling of newly synthesized proteins was performed by extending the incubation period for an additional 4 h. We labeled with 12.5 μCi of ^{35}S-methionine per ml of medium. The incubated medium was removed from culture flasks and stored. Since, the genes under study code for secreted proteins, we recovered their expressed products from 150 and 300 μl aliquots of the media by precipitating twice with four volumes of cold acetone. Subsequently, we dissolved the recovered proteins in layering buffer for SDS-polyacrylamide gel electrophoresis (21).

Southern blotting, Northern analysis, visualization of labeled proteins and radioimmunoanalysis

^{32}P-dCTP was purchased from Amersham Intl. (Buckinghamshire, England). Hybridization of DNA in nitrocellulose membrane was carried out as described by Southern (16). RNA was denatured and resolved according to size by agarose gel (22) electrophoresis. Once the above was performed, they were transferred to nitrocellulose sheets and hybridized to the probe (23). Both hybridization techniques used as probe, a 550 bp *Hae*III fragment of hPL cDNA (24) labeled with ^{32}P-dCTP by the technique of random primers (25).

Protein samples dissolved in layering buffer were boiled for 2 min and applied to 5−13% discontinuous polyacrylamide gels (21). Gels were placed on filter paper and dried under vacuum at 80°C. The dried gels were exposed to X-ray films at room temperature. Quantification of hGH was achieved using a commercially available hGH radioimmunoassay kit (Diagnostic products Co., Los Angeles, CA).

RESULTS

A new set of high expression plasmids for hPL structural genes

pNUT, contains the SV40 enhancer and metallothionein promoter directing the transcription of the promoter-less hGH-N gene (figure 1A). In addition, it efficiently expresses hGH in cell culture (12). We found, by radioimmunoassay, that COS-7 cells transfected with pNUT by the calcium phosphate method (18), yielded extracellular hGH values averaging 700 ng per 25 cm^2 culture flask.

We transferred the structural sequences (promoter-less) of all the hPL members of the hGH-hPL multigene family into pNUT (see figure 1B). This was accomplished by simply replacing the hGH-N gene structural sequences present in pNUT, for the corresponding sequences of the hPL genes. However, because hPL-1 gene lacks the convenient *Bam*HI site used for the transfer, we constructed a hybrid gene between hPL-1 and hPL-3 genes to provide it with such a site. The hybrid consists of the *Bam*H1−5′ end flanked first exon, first intron, and part of the second exon of the hPL-3 gene. The rest consists of hPL-1 sequences from the *Pvu*II site, within the second exon, to the *Eco*RI site at the 3′ end of the gene. Having constructed this hybrid allowed us to not only gain the useful *Bam*H1 site, but also allowed us to retain intact the second exon/second intron boundary of hPL-1. This area includes the donor splice mutation of interest, previously identified as potentially being the cause of lack of hPL-1 gene expression. From here on, this hybrid gene will be used instead of the hPL-1 wild-type gene. The recombinant plasmids were characterized by digestion with restriction enzymes (figure 1C) and by Southern blot analysis (figure 1D).

Expression of transfected hPL genes at the protein level

The figure 2 autoradiography reveals that cells transfected with the plasmid carrying the hGH-N structural gene (pNUT), secreted characteristic 22 kDa and 20 kDa forms of hGH (lane: hGH-N). The lane containing media from pNUThPL-1 transfected cells (lane: hPL-1), does not exhibit bands of at least the same intensity

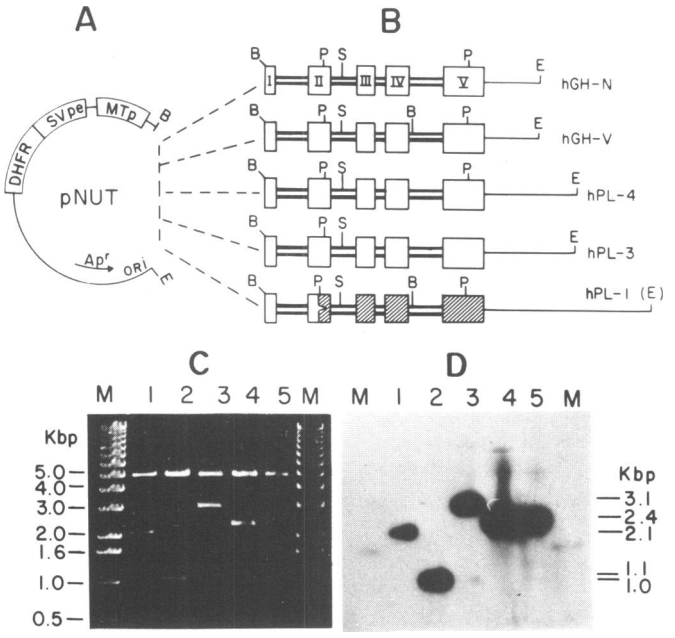

Figure 1. Construction of the hPL and hGH expression plasmids. To subclone hPL genes into pNUT(A), we replaced the hGH-N gene sequences in pNUT for the corresponding ones of hPL genes (B). The drawings at the top illustrate the maps of the expression plasmids (A and B). Both, restriction enzyme (C) and DNA hybridization (D) analysis, confirmed the identity of the new expression plasmids. Lanes in C and D show gel and Southern autoradiography of plasmids cut with *Eco*RI plus *Bam*HI and are as follows: 1, pNUT; 2, pNUThGH-V; 3, pNUThPL-1; 4, pNUThPL-3; and 5, pNUThPL-4. M corresponds to molecular weight (in kbp; at the left of C) DNA standards. Only the sizes of hybridizing bands are indicated (in kbp at the right of D). The hPL-1 is really a hybrid of hPL-3 and hPL-1 (see Materials and Methods section for explanation). The pNUThGH-V construct, presented here, was not analyzed. MTp=mouse metallothionein promoter; SVpe=SV40 early promoter; DHFR=structural gene for dihydrofolate reductase; Ap^r=B-lactamase gene; ORI=pBR322 origin of replication; B=*Bam*HI; P=*Pvu*II, S=*Sac*I, E=*Eco*RI. Boxes represent exons.

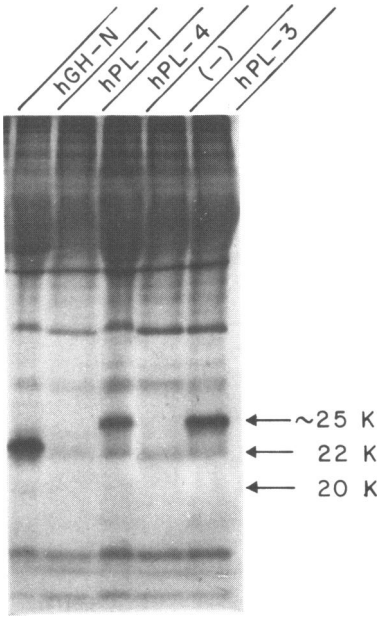

Figure 2. *In vitro* production of secreted proteins by hGH-N and hPL genes. Media from COS-7 cells transfected with each of the plasmids and incubated in the presence of ^{35}S-methionine, was analyzed by discontinuous SDS-polyacrylamide gel (5%−13%) electrophoresis and autoradiography. The gene present in each plasmid used for transfection is indicated at the top. The (−) symbol identifies the media from cells transfected with the vector alone [pNUT(−)]. Sizes of characteristic hPL and hGH bands are indicated in kDa (K) at the left.

and size of hGH. Media from cells transfected with pNUThPL-4 (lane: hPL-4) presents a less prominent band, but of slightly greater size (close to 25 kDa) than that of hGH. Finally, the only hPL-3 form observed (lane: hPL-3), is of hPL-4 size. However, its intensity is that of the 22 kDa form of hGH.

Differences in the expression of hPL proteins

As noticed above (figure 2), while the hGH-N gene gives rise to a prominent band of approximately 22 kDa, hPL-3 and hPL-4 genes express proteins of slightly greater size (~25 kDa). Furthermore, and consistently throughout several independent experiments, the hPL-4 band always appeared weaker than the hPL-3 band. We were interested in investigating the cause of such heterogeneity in the expression levels of these genes.

To approach this problem, we chose to study the cell culture production of extracellular hPL-hGH chimeric proteins resulting from the transient expression of a new hybrid gene pair. These hybrids possess the first two exons from hPL-3 or hPL-4 genes, and sequences of hGH-N gene that conform the remaining part of their structure. We named these hybrids GH(PL-3:I,II) and GH(PL-4:I,II) respectively (see map in figure 3A). By comparing both hybrid genes, differences were observed in the expression levels of GH(PL-3:I,II) versus GH(PL-4:I,II) chimeric proteins [figure 3B; compare lanes labelled GH(PL-3:I,II) and GH(PL-4:I, II)]. Same results, we might add, as with the proteins derived from normal non-hybrid hPL-3 and hPL-4 genes (see lanes labeled hPL-3 and hPL-4 in figure 2 and labeled PL-3 and PL-4 in figure 3B).

Consistently, we obtained less protein when sequences from the first two exons of hPL-4 gene were present. On the contrary, we detected more hGH-like protein when the first two exons were from hPL-3. Moreover, when the last three exons were from hPL-4 gene, and the first two exons were from hGH-N (see map of this chimeric at bottom of figure 3A), the band intensity resembled that of hGH-N protein [figure 3B, lane PL-4(GH:I,II)]. Therefore, relative abundance of hPL protein products is a function of the first two exons.

To further investigate the different levels of *in vitro* expression observed for these active hPL genes, we carried out estimations of the relative abundance of their RNA transcripts. Using slot blot analysis, we found approximately ~8-fold more RNA hybridizable to our probe from the total RNA isolated of cells transfected with the hPL-3 structural gene sequences, as compared to hPL-4 (figure 4). This same result was observed even when only the two first exons of hPL-3 were contributing to a hybrid gene (D.R.-P. and H.A.B.-S., submitted. Therefore, the higher observed hPL-3 protein expression, seems to be a consequence of having more RNA derived from the hPL-3 gene.

Dissecting the putative hPL-1 pseudogene

Next we, decided to test if the donor splice site point mutation at the second intron of the hPL-1 gene, was the only cause of the apparent inactivity of this gene. Comparing nucleotide sequences at the second exon/second intron border area, among the active hPL-3 gene and the putative hPL-1 pseudogene, revealed that we could easily exchange this region between these two genes. We found *Pvu*II and *Sac*I sites located 30 bp upstream and 86 bp downstream respectively, from the mutation site (figure

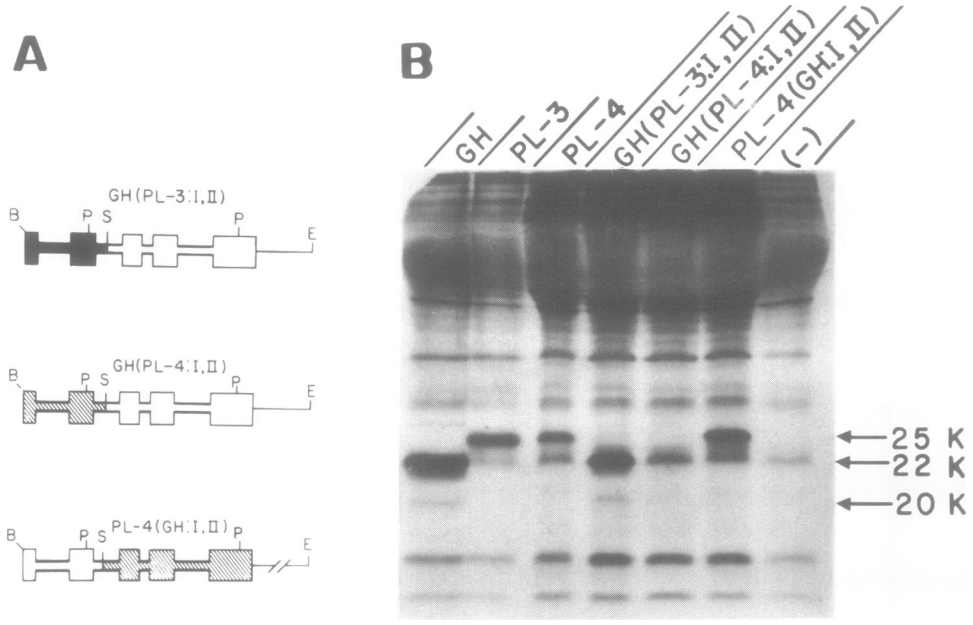

Figure 3. Diversity in size and abundance of protein expression products from, hPL and recombined hPL-hGH genes. Maps in A demonstrates the structure of the hybrid genes; their are composed of hPL-3 (solid) or hPL-4 (hatched) and hGH (open) gene portions. As in the previous figures, dealing with protein analysis, each lane in B corresponds to media of cells transfected with plasmids carrying the genes indicated at the top. The negative control, (−) symbol, distinguishes the media from cells transfected with pNUT(−) (vector alone). The new bands are pointed by arrows. Size is also in kDa (k).

1B). In addition to the splice site point mutation, this region flanked by *Pvu*II and *Sac*I of approximately 120 bp, differs within these two genes in only three nucleotide positions. These differences, are located inside the intron at nucleotide positions of little importance for the pre-mRNA processing (26). The *Pvu*II-*Sac*I region encompassing the second exon/second intron boundary, was exchanged between the putative hPL-1 pseudogene and the active hPL-3 gene. This manipulation gave rise to two new hybrid genes. We named these new recombinants as hPL-1r and hPL-3m, for 'repaired' hPL-1 and 'mutated' hPL-3 genes, respectively.

To determine the effect on the splice mutation expression, we transferred these hybrid genes, once constructed, into pNUT. Both the hybrid and non-hybrid genes (controls) were introduced into COS-7 cultured cells. Figure 5B demonstrates the results obtained from the analysis of *in vivo* labeled secreted proteins. In lane labeled PL-3 which corresponds to media of cells transfected with pNUThPL-3, we easily detected an hPL band. On the other hand, cells transfected with either pNUThPL-1 (lane: PL-1) or pNUThPL-3m (lane: PL-3m), revealed the absence of obvious hPL bands. Likewise, we could not observe an hPL band from the media of cells transfected with the new repaired hPL-1 gene (figure 5B, lane: PL-1r).

A dramatic effect of the splice donor site mutation at the second intron of hPL-1 was also observed; when, we constructed and used in comparative studies, a new hybrid gene. It consists of hPL-3m sequences, joined at the unique *Sac*I site (within the second intron) to the remaining portion of hGH-N gene (bottom of figure 5A). This new hybrid was studied in conjunction (as positive control) with the previously mentioned hybrid gene created between hPL-3 and hGH-N.

A prominent hGH-like (similar in hGH size) protein was found in the media of cells transfected with the non-mutant hybrid gene [see map of GH(PL-3:I,II) in figure 5A, and expression results in lane: GH(PL-3:I,II) of figure 5B]. This positive control gene also carries the alternative splice acceptor site present inside exon 3 of the hGH-N gene (4). Splicing exons 1 and 2 to this alternative acceptor site, results in the 15 amino acid internal deletion characteristic of the 20 kDa form of hGH. The presence of a new minor band of 20 kDa together with the 22 kDa band, in the media of cells transfected with pNUTGH(PL-3:I, II), demonstrates that the alternative splicing mechanism also occurs in this hybrid gene.

No hPL or hGH-like proteins were observed (last lane in figure 5B) when the second member of this pair carrying the hPL-1 mutation was used in the transfection.

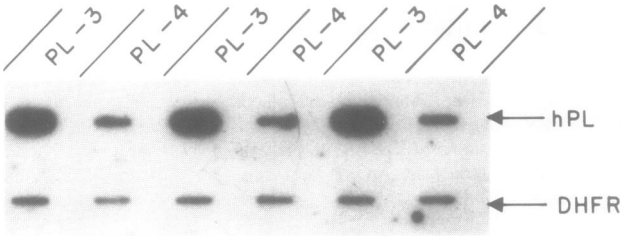

Figure 4. Slot Blot analysis of hPL-3 and hPL-4 RNA expressions. Total cellular RNAs were obtained from three independent experiments in which COS-7 cells were transfected with either pNUThPL-3, or pNUThPL-4. 3μg of RNAs were applied to the slots and hybridized with a DHFR cDNA probe. To determine efficiencies of transfection, the slots were cut and its radioactivity contents measured by liquid scintillation counting. RNAs in amounts compensating transfection efficiencies, were then hybridized separately with the DHFR and with an hPL cDNA probe (arrows). Extent of hybridization was also assessed by counting radioactivity in each slot.

Effect of the splice mutation of hPL-1 gene in mRNA production

We wanted to know whether the apparent absence of hPL protein from cells transfected with the pNUThPL-1 plasmid, was a

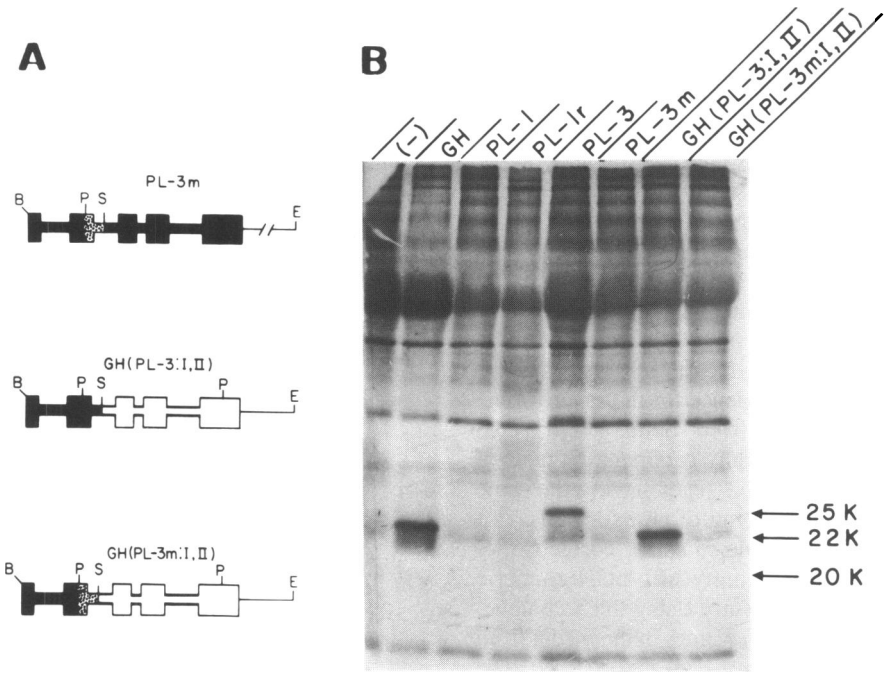

Figure 5. Effect of the donor splice site mutation of hPL-1 gene on the production of secreted proteins. Cells transfected with the different plasmid carrying the hybrid genes indicated at the top, were analyzed for the presence of secreted labeled proteins in their media. Hybrid genes were constructed as described in the Material and Methods and Results sections, and for purpose of clarification, maps of key hybrid genes are shown in A. Characteristic non-chimeric and chimeric proteins were visualized as described in Material and Methods section, gene portions in black, hPL-3 gene; stippled, hPL-1; and open, hGH. Only important recognition sites for restriction enzymes are indicated, B=*Bam*H1, P=*Pvu*II, S=*Sac*1 and E. *Eco*RI. Arrows in B indicate the size in kDa (k) of the new proteins originated from plasmids transfected. (−)=media from the negative control cells.

consequence of having no RNA derived from the PL-1 gene. We performed a Northern blot analysis (23) of the total RNA extracted from transfected cells. We included as a positive control, RNA isolated from human placenta and pituitary gland. In addition, as another positive control, we included RNA from cells tranfected with the plasmid carrying the hPL-3 structural sequences. The results of this analysis are presented in figure 6. Cells transfected with the plasmid carrying the hPL-1 gene lacked hPL specific RNAs (figure 6, lane: PL-1). We observed a dramatic reduction in the hybridizable RNA content from cells transfected with the plasmid carrying the hPL-3 mutated gene (figure 6, lane: PL-3m); as compared with, the wild type hPL-3 gene acting as control (lane: PL-3). Finally, when using pNUThPL-1r, we unexpectantly observed only a faint reappearance of hPL mRNA (figure 6, lane: PL-1r).

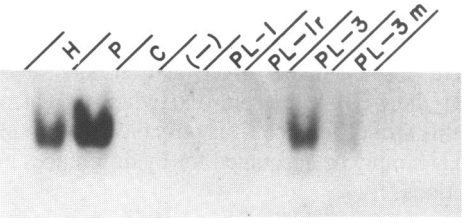

Figure 6. RNA expression effect of the donor splice site mutation of hPL-1 gene. The figure demonstrates the results of the Northern blot analysis (23) practiced to total RNA (10 μg), isolated from cultured cells transfected with the indicated (at top) plasmids. H and P represent lanes containing total RNA from human pituitary gland (2 μg) and placenta (3.8 μg), respectively. The (−) symbol correspond to RNA (10 μg) isolated from cells transfected with the pNUT (vector alone). Lane labeled C represents total RNA of mock-transfected cells.

DISCUSSION

DNA cloning and sequence studies have lead to the isolation of cDNA clones for all hGH and hPL genes except hPL-1. However, experiments directed to demonstrate that each of the mRNAs corresponding to the identified cDNAs indeed end up as proteins, have been few for hGH (7, 27) and none for hPL genes. The reintroduction of cloned genes into cultured cells, by DNA transfection (18), is a valuable method to identify and dissect sequences required for gene function and their mutations. We chose this approach to determine the coding potential of all hPL genes. To achieve our objective, we forced the *in vitro* expression of all the hPL promoter-less genes, by joining them to the strong heterologous transcriptional control sequences present in pNUT (12).

The new results of the present study demonstrate for the first time that the hPL-4 and hPL-3 genes, but not hPL-1, contribute to the production of mature hPL. Here we also demonstrate that in spite of being highly similar, the structural sequences of these genes respond differently to the same heterologous promoter. Each of these two genes give rise to one protein. Although the secreted proteins expressed by these genes have identical amino acid sequence, they differ at their expression level. As a consequence of this finding, we designed exon exchange experiments to gain new insights in the understanding of this phenomenon. The same result was seen when only the two first exons of the genes were contributing to a hybrid gene. The observed higher hPL-3 protein expression seems in part to be a consequence of having more RNA expression from the hPL-3 gene sequences. This *in vitro* findings do not resemble what occurs *in vivo*, while the hPL-4 mRNA (HCS-A) accounts for

3% of the placenta mRNA, the hPL-3 (HCS-B) mRNA has been estimated to represent only 0.5% of it (28).

Finally, a third contribution of our study is the experimental demonstration, also for the first time, of the effect of the donor splice mutation at the second intron of hPL-1 on its expression. In spite of having replaced the donor splice site mutation at the second intron of the hPL-1 pseudogene by normal sequences, we could not observe an hPL-1 protein secreted into the cell medium. Thus, there must exist additional mutations that contribute to this lack of genetic expression. There is no doubt of the severity of this splicing mutation. Indeed we prove here that by introducing the mutated area of hPL-1, into either the hPL-3 or the hGH-N gene, protein production from these hybrid genes is severely reduced. Further evidence for the severe effect of this type of mutation comes from both, site-directed mutagenesis studies (26) and by naturally occurring mutants of β-globin genes. In both cases, mutated genes having the same change of a G for an A at the beginning of an exon, give rise to a messenger RNA precursor unable to splice correctly.

We recently found that we can quantify hPL-hGH chimeric proteins using an hGH radioimmunoassay. Using this technique, non-detectable hGH radioimmunoassay values in the media from cells transfected with pNUTGH(PL-3m:I,II) were observed. The media of cells corresponding to the control experiment, where GH(PL-3:I,II) gene was used, gave hGH RIA values of about half of those of pNUT. Five-fold lower RIA values were found for the expression of GH(PL-4:I,II) as compared to GH(PL-3:I,II) (D.R-P., and H.A.B-S, submitted).

We have no explanation for the difference in size observed in the electrophoretic analysis of hGH and hPL proteins. The difference was seen with both purified hormones from the pituitary gland and placenta, and with the proteins produced in the gene transfection experiments. Neither hPL-3 nor hPL-4 proteins have the N-linked glycosylation site at Asn-140, predicted for the hGH-V protein and which might otherwise account for this size difference. It is possible that the size change observed, may simply be accounted for by differences in protein-SDS interactions.

In conclusion, our analysis of the *in vitro* expression products of hPL genes demonstrates that hPL-3 and hPL-4 have the potential for contributing to mature hPL. It also provides evidence indicating that the hPL-1 gene has accumulated severe mutation(s), other than the donor splice site deffect at its second intron. Furthermore, through our study, we have identified the gene region between the capping site and the second intron as the origin of differences in expression levels seen here for hPL-3 and hPL-4. The results obtained with the hPL-hGH genes hybrids corroborate and strengthen our findings.

ACKNOWLEDGEMENTS

We thank A. Martínez for assistance with some of these experiments, J. Silva for proofreading and critical reading and D. Baty for reviewing the manuscript. We also thank Dr. T. Kuo for providing the COS-7 cells and donation of materials, Dr. G. Saunders for the generous gift of plasmids and for reviewing the manuscript and Dr. E. Olson the donation of ^{35}S-Methionine. We are grateful to Dr. R. Palmiter for providing the pNUT plasmid and also for his interest and helpful suggestions. R.R.S., D.R.P. and A.V.E. were recipients of scholarships from the Consejo Nacional de Ciencia y Tecnología (CONACYT) of the Mexican government. This work was supported by grants from CONACYT, the Subsecretaría de Educación Superior e Investigación Científica de la Secretaría de Educación Pública of the Mexican government, Centro Internacional de Biología Molecular y Celular, A.C., and from General Foods and IBM of Mexico. We are indebted to G. García-L., G. Infante-García and S. Silva-Herrera from the Servicio de Endocrinología del Hospital Universitario 'Dr. José E. González' for their aid with the radioimmunoassays, and to M. Páez for preparation of the manuscript.

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ABBREVIATIONS

bGH, bovine growth hormone; bp, base pairs; CAT, chloramphenicol acetyl transferase; FCS, fetal calf serum; hCS, human chorionic somatomammotropin; hGH, human growth hormone; hPL, human placental lactogen; hPrl, human prolactin; kbp, 1000 base pairs; kDa, 1000 daltons; Pre-hPL, hPL precursor; SV40, simian virus 40; SDS, sodium dodecyl sulphate.